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(71) Applicant *(for all designated States except US)*: **DI-ACHIP CO., LTD.** [KR/KR]; 1510-8 Bongcheon 7-dong, Gwanak-gu, Seoul 151-057 (KR).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **HWANG, Young, Min** [KR/KR]; Tae-jayon Apt. 102-604, Kwanjeo-dong, Seo-ku, Taejon 302-724 (KR).

(74) Agent: **JANG, Seong, Ku**; 17th Fl., KEC Building, 275-7, Yangjae-dong, Seocho-ku, Seoul 137-130 (KR).

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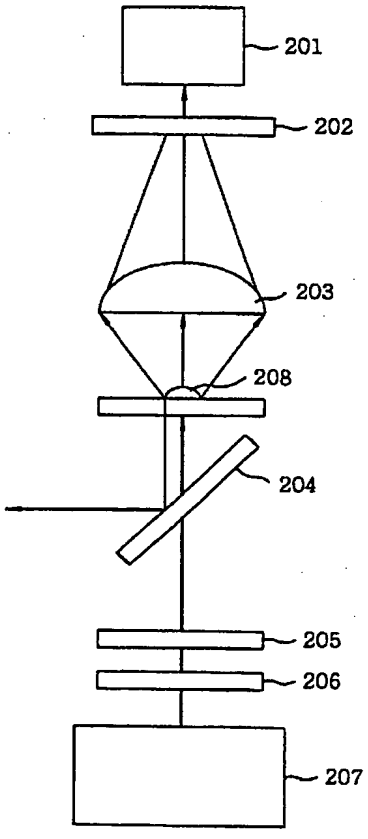
(54) Title: APPARATUS FOR ANALYZING FLORESCENT IMAGE OF BIOCHIP



(57) Abstract: An apparatus for analyzing a fluorescent image of a biochip includes a light source for generating excitation light, a diffusion filter for diffusing the excitation light, an excitation filter for filtering light having a wavelength within a first spectrum range out of the excitation light, a beam splitter for filtering the excitation light to be irradiated on the sample and filtering out light having a wavelength within a second spectrum range out of the light emitted from the sample. By using the beam splitter for filtering through the light from the excitation light source and filtering out the light emitted from the sample excited by the excitation light, the apparatus can irradiate light having a constant intensity on a wide area of a biochip.

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APPARATUS FOR ANALYZING FLUORESCENT IMAGE OF BIOCHIP

Technical Field

The present invention relates to a fluorescent image analyzer for detecting light having a wavelength within a second spectrum range emitted from a sample on a biochip excited by light having a wavelength within a first spectrum range; and, more particularly, to a fluorescent image analyzer capable of directly eradiating light having a wavelength within a first spectrum range onto a sample on a biochip by using a beam splitter for filtering through the light having a wavelength only within the first spectrum range but reflecting light having a wavelength within a second spectrum range emitted from the sample, thereby enabling a fluorescent image of the biochip to be efficiently analyzed.

Background Art

A biological chip or a biochip is also called as a biological array and the concept thereof has been recently introduced. The biochip is obtained by fixing on a substrate a biological material such as nucleic acid. Two types of biochips are well known: a DNA chip with DNA fixed on a substrate and a proteinic chip with protein fixed on a substrate. In some cases, RNA may be fixed on a substrate to be referred to as an RNA chip.

A basic principle of the biochip is grounded on interactions between fixed molecules on a substrate and target molecules. For example, the principle of the DNA chip is based on a complementary connection between oligonucleotides fixed on a substrate and a DNA base existing in a sample. As another example, the principle of the proteinic chip is based on an interaction between

proteinic molecules such as an antigen-antibody connection or a ligand-receptor connection.

A biochip is conventionally analyzed and examined as follows. A target molecule having a fluorescent material attached thereto is transferred to a fixed molecule on a substrate, so that a reaction between the target molecule and the fixed molecule is induced. Thereafter, the fluorescent material is excited by light whose wavelength belongs to a certain spectrum range. Then, light having a wavelength that falls within another spectrum range is filtered out of light emitted from the excited fluorescent material. In case of determining whether an AIDS virus antibody exists in human blood, for example, a biochip with antigens separated from an AIDS virus arranged thereon is prepared and a serum sample and a secondary antibody having a fluorescent material attached thereto are continuously transferred to every antigen spot on the biochip, thereby triggering a reaction between the AIDS virus antigens and the secondary antibody. After the reaction is completed, the biochip is analyzed quantitatively or qualitatively by using a fluorescent reader. In other words, the conventional biochip analysis method as described above employs the fluorescent material based on the fact that internal energy of the fluorescent material is excited to a higher level if it is exposed to light having a certain wavelength, i.e., an excitation wavelength, and emits light having a wavelength longer than the excitation wavelength at a time when the once increased internal energy is lowered to an original level.

In general, an apparatus for analyzing a fluorescent image of a biochip includes an excitation light source for generating excitation light and a sensor for detecting light within a certain spectrum range emitted from a sample excited by the excitation light. Widely employed as the excitation light source are a Xenon lamp, a Mercury lamp, an

Argon-ion lamp, a YAG laser, and a HeNe laser. As the sensor of the fluorescent image analyzer for the biochip, a PMT (photomultiplier tube) sensor, a CCD (charge-coupled device) camera or a cooled CCD camera is commonly utilized. The PMT sensor estimates an intensity of inputted light and generates an output current in proportion to the intensity of the inputted light. The PMT sensor is so sensitive that it can count each photon of the light. The CCD camera has an advantage in that it can detect light within a wide range at a time, which is different from the PMT sensor. In general, the Cooled CCD is more widely employed in a fluorescent image analyzer for a biochip than the CCD camera because it exhibits a higher sensitivity than that of the CCD camera.

Referring to Fig. 1, there is illustrated a conventional fluorescent image analyzer such as a scanner for obtaining an image by filtering through light emitted from a fluorescent material and a microscope having a camera attached thereto.

In general, a laser or a filament bulb is utilized as an excitation light source 101 in the fluorescent image analyzer. The laser is particularly advantageous since it enables to obtain light having a single wavelength without involving a filtering process. In this fluorescent image analyzer, excitation light is eradiated on a sample 106 via an object lens 102. Then, light that falls within a certain angular range with respect to an eradiation direction of the excitation light is collected by the object lens 102 out of light emitted from the sample. At this time, since the fluorescent light emitted from the sample 106 is radiated in all directions, it is very important to properly adjust a condensing angle of the object lens 102. Since the intensity of the light from the excitation light source 101 is much larger than that of the fluorescent light emitted from the sample 106, there is demanded a method for

distinguishing a wavelength of the fluorescent light from a wavelength of the light from the excitation light source 101 in order to receive at a detector 105 only the fluorescent light emitted from the sample 106 without being affected by the light from the excitation light source 101. For the purpose, a beam splitter 103 such as a dichroic filter is employed to reflect the light from the excitation light source 101 but filter through the fluorescent light emitted from the sample 106. Further, an emission filter 104 is installed in front of the detector 105 so as to allow only the light having a wavelength within a certain spectrum range to reach the detector 105. A PMT sensor or a CCD camera is used as the detector 105.

As described above, the conventional fluorescent image analyzer has a structure in which the light from the excitation light source 101 is radiated from a lateral side of the analyzer and is reflected by the beam splitter 103 in a predetermined angle to be eradiated onto the sample 106. However, the intensity of the light emitted from the fluorescent material is linearly proportional to the intensity of the light received from the excitation light source 101. Thus, it will be more effective for the analysis of the fluorescent image to shorten a distance from the excitation light source 101 to the detector 105. Further, the light from the excitation light source needs to be directly eradiated to the sample in order to apply light having a constant intensity to an analysis area on the sample.

Disclosure of Invention

It is, therefore, an object of the present invention to provide an apparatus for analyzing a fluorescent image of a biochip by using an excitation light source for directly eradiating light on a sample on the biochip and a beam

splitter for filtering through light having a wavelength only within a first spectrum range from the excitation light source but reflecting light having a wavelength within a second spectrum range emitted from the sample excited by the light from the excitation light source, thereby improving the efficiency of the light eradiated on the sample.

It is another object of the preset invention to provide an apparatus for analyzing a fluorescent image of a biochip by using an excitation light source capable of uniformly and efficiently eradiating light onto an analysis area of the biochip.

In accordance with a preferred embodiment of the present invention, there is provided an apparatus for analyzing a fluorescent image of a biochip including: a light source for eradiating light having a wavelength within a first spectrum range on a sample on the biochip; a beam splitter positioned between the biochip and the light source for filtering through the light only within the first spectrum range but reflecting light having a wavelength within a second spectrum range emitted from the sample excited by the light from the light source; an object lens for collecting the light within the second spectrum range; an emission filter for filtering through only the light within the second spectrum range; and a detector for detecting the light within the second spectrum range filtered through by the emission filter.

In accordance with another preferred embodiment of the present invention, there is provided an apparatus for analyzing a fluorescent image of a biochip including: a plate-type light source made of an array of chip LEDs for eradiating light having a wavelength within a first spectrum range on a sample on the biochip; a beam splitter installed between the biochip and the light source for filtering through the light only within the first spectrum range but reflecting light having a wavelength within a second

spectrum range emitted from the sample excited by the light from the light source; an object lens for collecting the light within the second spectrum range; and a detector for detecting the light within the second spectrum range collected by the object lens.

Brief Description of Drawings

The above and other objects and features of the invention will become apparent from the following description of preferred embodiments given in conjunction with the accompanying drawings, in which:

Fig. 1 is a block diagram of a conventional fluorescent image analyzer;

Fig. 2 offers a block diagram of a fluorescent image analyzer in accordance with a first preferred embodiment of the present invention;

Fig. 3 sets forth a graph for illustrating spectrums from an emission filter, an excitation filter and a beam splitter in accordance with the present invention;

Fig. 4 illustrates a block diagram of a fluorescent image analyzer in accordance with a second preferred embodiment of the preset invention; and

Fig. 5 depicts a block diagram of a fluorescent image analyzer in accordance with a third preferred embodiment of the present invention.

Best Mode for Carrying Out the Invention

Referring to Fig. 2, there is illustrated a block diagram of a fluorescent image analyzer in accordance with a first embodiment of the present invention.

As shown in Fig. 2, the fluorescent image analyzer has an excitation light source 207 at a bottom portion thereof. This structure allows light from the excitation light source

207 to be directly eradiated onto a sample 208. The excitation light source 207 herein used may be a laser, a lamp or a chip LED (light emitting diode). As the excitation light source 207, the laser has an advantage in that stable light featuring a large output for a certain wavelength can be obtained such that a detection limit is lowered. The laser, however, also has a disadvantage since it is very difficult to uniformly apply laser beams to a wide area on the sample and the price thereof is very high.

A diffusion filter 206 may be used in the fluorescent image analyzer in order to allow the light from the excitation light source 207 to be uniformly eradiated on an analysis area on the sample 208. A plate-type light source made of an array of chip LEDs can also be utilized as the excitation light source 207 for the uniform eradiation of the excitation light onto the sample 208. Furthermore, the excitation light source 207 may have a collimator (not shown) for making diverging beams outputted therefrom parallel to each other.

As can be seen from Fig.2, the diffusion filter 206 can be disposed in a direction in which the excitation light is outputted from the excitation light source 207. The diffusion filter 206 allows the excitation light to be radiated on the sample 208 at a constant intensity. If the excitation light source 207 does not generate light having a single wavelength such as a laser beam, an excitation filter 205 may be used to extract light within a certain spectrum range from the excitation light.

As mentioned before, a beam splitter or a dichroic filter used in the conventional fluorescent image analyzer operates to reflect light having a wavelength within a certain spectrum range radiated from the excitation light source but filter through light having a wavelength within another spectrum range emitted from the sample. A beam splitter 204 in the first embodiment of the present

invention, however, has a reverse function. In other words, the beam splitter 204 allows the light having a wavelength within a certain spectrum range provided from the excitation light source 207 to pass therethrough but reflects the light having a wavelength within another spectrum range emitted from the sample 208. Accordingly, it can be prevented that the light from the sample 208 is reflected by the excitation filter 205 after passing through the beam splitter 204 to be inputted to a detector 201 again via the beam splitter 204 and an object lens 203, resulting in an overlapped fluorescent image at a detector 201. Further, by using the beam splitter 204 of the present invention, the light from the excitation light source 207 can be directly radiated onto the sample 208 without being refracted.

As shown in Fig. 2, installed right above the sample 208 is the object lens 203 for collecting fluorescent light emitted from the sample 208 and transmitting the collected fluorescent light to the detector 201. Further, prepared between the object lens 203 and the detector 201 is an emission filter 202 for filtering through the light having a wavelength only within a certain spectrum range out of the light emitted from the sample 208. The detector 201 may be a PMT or a Cooled CCD.

Fig. 3 illustrates spectrums from the beam splitter 204, the excitation filter 205 and the emission filter 202. Solid lines 302, 304 and 305 represent spectrum ranges of light transmitted through the emission filter 202, the beam splitter 204 and the excitation filter 205, respectively. Further, an area defined by oblique lines refers to a spectrum range of light absorbed by the sample 208 while a checkered area represents a spectrum range of the light emitted from the sample 208 excited by the excitation light from the light source 207.

A more detailed description of a relationship between the spectrums shown in Fig. 3 will be provided as follows.

The beam splitter 204 only filters through light having a wavelength only less than a specific value (e.g., 500 nm) out of the light from the excitation light source 207. Accordingly, light having a long wavelength (e.g., longer than 500 nm) emitted from the sample 208 cannot be transmitted through the beam splitter 204. Thus, it can be prevented that an overlapped fluorescent image is detected at the detector 201. The excitation filter 205 filters through light having a wavelength that falls only within a certain range (e.g., ranging from 450 to 500 nm) out of the light coming from the excitation light source 207. The emission filter 202 filters through light having a wavelength that belongs to only a specific range (e.g., ranging from 500 to 550 nm) out of the light collected by the object lens 203 after emitted from the sample 208. At this time, the emission filter 202 prevents the light from the excitation light source 207 from reaching the detector 202 together with the light from the sample 208. Accordingly, only the fluorescent light from the sample 208 can reach the detector 201 to be detected thereat.

Fig. 4 illustrates a fluorescent image analyzer in accordance with a second embodiment of the present invention.

A detector 401 is installed at a lateral side of the fluorescent image analyzer. Light radiated from an excitation light source 407 is selected to reach a sample 408 only if its wavelength falls within a first spectrum range and light emitted from the sample 408 is allowed to reach the detector 401 only if it has a wavelength falling within a second spectrum range. The emitted light is reflected by a beam splitter 404 and collected by an object lens 403 to be detected at the detector 401. Installed between the object lens 403 and the detector 401 is an emission filter 402 for preventing light having a wavelength that falls out of a second spectrum range from reaching the detector 401. Further, as described in the first embodiment

shown in Fig. 2, a diffusion filter 406 and/or an excitation filter 405 can be disposed between the excitation light source 407 and the beam splitter 404, if necessary, in order to select the light having a wavelength only within the first spectrum range to thereby allow it to reach the sample 408 at a constant intensity.

A plate-type light source made of an array of chip LEDs can be utilized as the excitation light source 407. Further, a collimator (not shown) can be prepared at the excitation light source 407, if necessary, so as to make diverging beams outputted from the excitation light source 407 parallel to each other.

Referring to Fig. 5, there is shown a fluorescent image analyzer in accordance with a third embodiment of the present invention.

As in the second embodiment shown in Fig. 4, a detector 501 is installed at a lateral side of the fluorescent image analyzer. Unlike in the second embodiment, however, an additional excitation light source 512 is installed at a bottom portion of the analyzer in addition to an excitation light source 507 prepared at a top portion thereof in Fig. 5. By using the two excitation light sources 507 and 512, excitation light having a greater intensity can be applied to a sample 508, so that a more effective analysis of a fluorescent image can be conducted.

Light radiated from the excitation light sources 507 and 512 is allowed to reach the sample 508 only if its wavelength falls within a first spectrum range and light emitted from the sample 508 is selected to reach the detector 501 only if it has a wavelength that belongs to a second spectrum range. The emitted light is reflected by a beam splitter 504 and then collected by an object lens 503 to be detected by the detector 501. Installed between the object lens 503 and the detector 501 is an emission filter 502 for preventing light having a wavelength that falls out

of the second spectrum range from reaching the detector 501. Further, as described before in the first preferred embodiment shown in Fig. 2, a diffusion filter 506 and/or an excitation filter 505 can be installed between the excitation light source 507 and the beam splitter 504 and likewise, a diffusion filter 511 and/or an excitation filter 510 can be installed between the excitation light source 512 and the beam splitter 509, thereby making the light having a wavelength only within the first spectrum range illuminate onto the sample 508 at a constant intensity.

Plate-type light sources made of an array of chip LEDs can be utilized as the excitation light sources 507 and 512. Further, a collimator (not shown) can be prepared at each of the excitation light sources 507 and 512, if necessary, so as to make diverging beams outputted therefrom parallel to each other.

In accordance with the present invention, a fluorescent image analyzer for a biochip employs an excitation light source for directly radiating light having a wavelength only within a first spectrum range onto a sample on the biochip and a beam splitter for filtering through the light from the excitation light source but reflecting light having a wavelength within a second spectrum range emitted from the sample. Therefore, light from a light source can be efficiently eradiated onto the sample at a constant intensity.

In addition, since the fluorescent image analyzer of the present invention utilizes a diffusion filter and/or a plate-type light source formed of an array of chip LEDs, it is possible to uniformly eradiate excitation light to a wide area on the sample as well as a small area thereon.

While the invention has been shown and described with respect to the preferred embodiments, it will be understood by those skilled in the art that various changes and modifications may be made without departing from the spirit

and scope of the invention as defined in the following claims.

CLAIMS

1. An apparatus for analyzing a fluorescent image of a biochip comprising:

a light source for eradiating light having a wavelength within a first spectrum range on a sample on the biochip;

a beam splitter positioned between the biochip and the light source for filtering through the light only within the first spectrum range but reflecting light having a wavelength within a second spectrum range emitted from the sample excited by the light from the light source;

an object lens for collecting the light within the second spectrum range;

an emission filter for filtering through only the light within the second spectrum range; and

a detector for detecting the light within the second spectrum range filtered by the emission filter.

2. The apparatus of claim 1, wherein the light source is a plate-type one made of an array of chip LEDs (light emitting diodes).

3. The apparatus of claim 1, further comprising a diffusion filter installed between the light source and the biochip for making the light from the light source illuminate onto the sample at a constant intensity.

4. The apparatus of claim 1, further comprising an excitation filter for filtering through only the light from the light source whose wavelength falls within the first spectrum range.

5. An apparatus for analyzing a fluorescent image of a biochip comprising:

a plate-type light source made of an array of chip LEDs for eradiating light having a wavelength within a first spectrum range to a sample on the biochip;

a beam splitter installed between the biochip and the light source for filtering through the light whose wavelength falls only within the first spectrum range but reflecting light having a wavelength within a second spectrum range emitted from the sample excited by the light from the light source;

an object lens for collecting the light within the second spectrum range; and

a detector for detecting the light within the second spectrum range collected by the object lens.

6. The apparatus of claim 5, further comprising an emission filter located between the biochip and the detector for filtering through only the light whose wavelength falls within the second spectrum range.

7. The apparatus of claim 5, further comprising a diffusion filter disposed between the biochip and the detector for making the light from the light source illuminate onto the sample at a constant intensity.

8. The apparatus of claim 5, further comprising an excitation filter for filtering through only the light from the light source whose wavelength falls within the first spectrum range.

FIG. 1

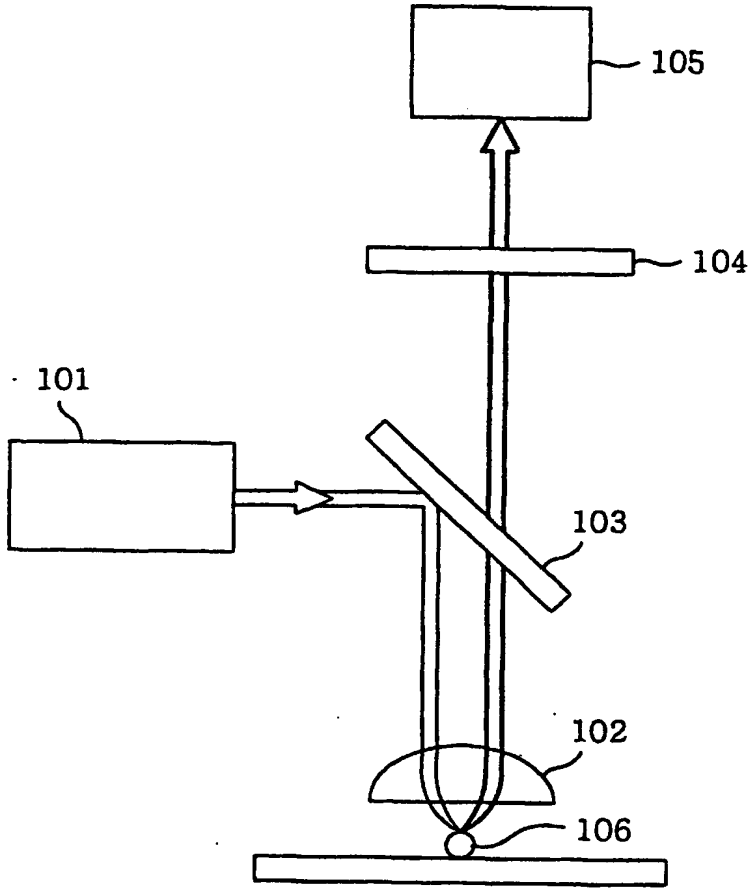


FIG.2

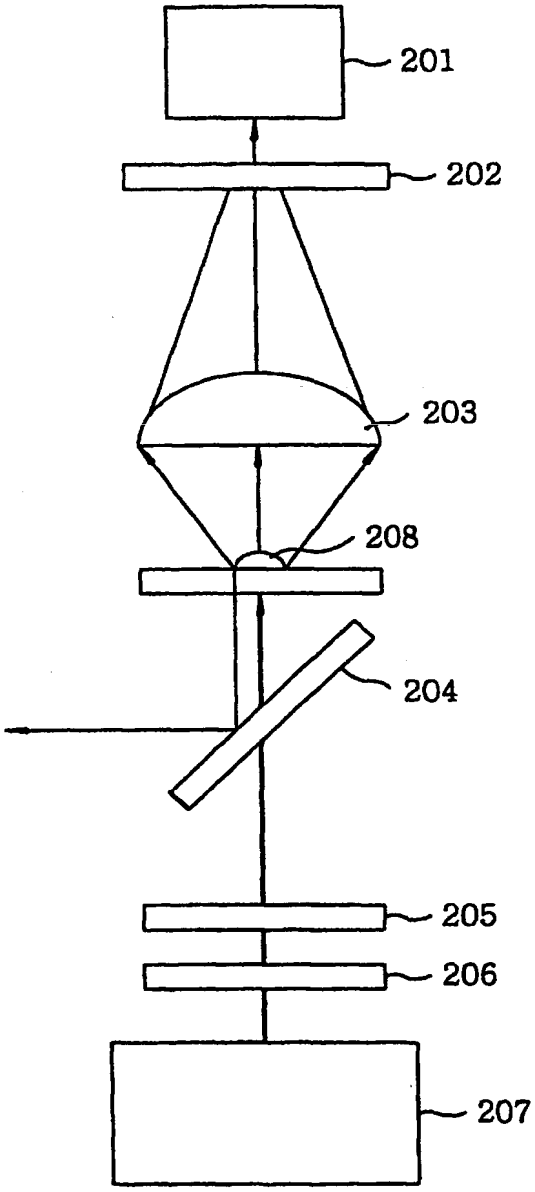


FIG.3

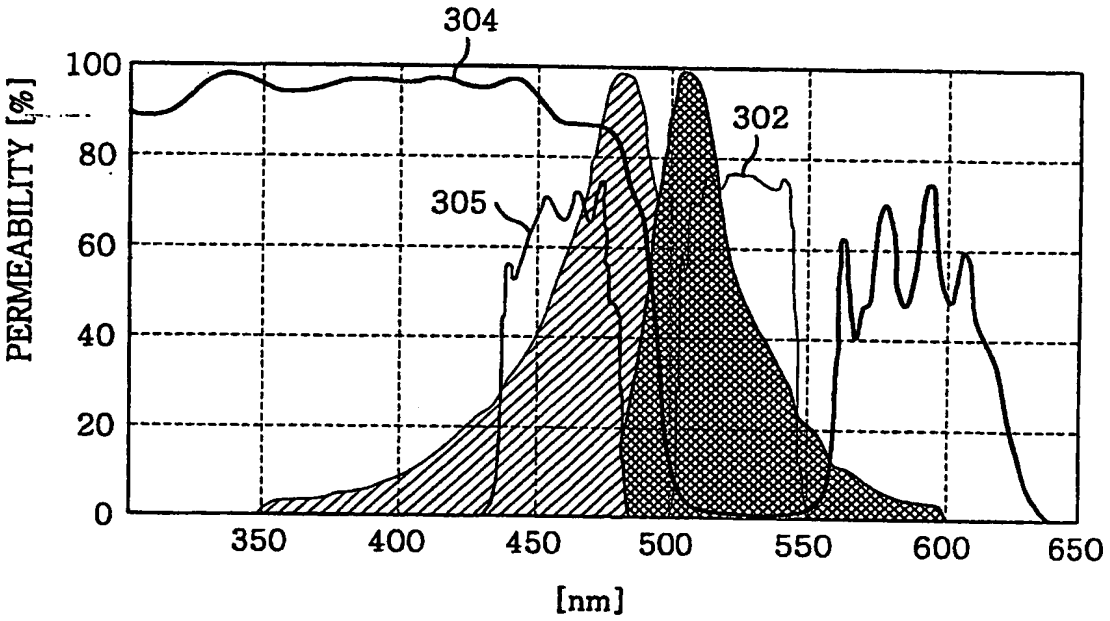


FIG. 4

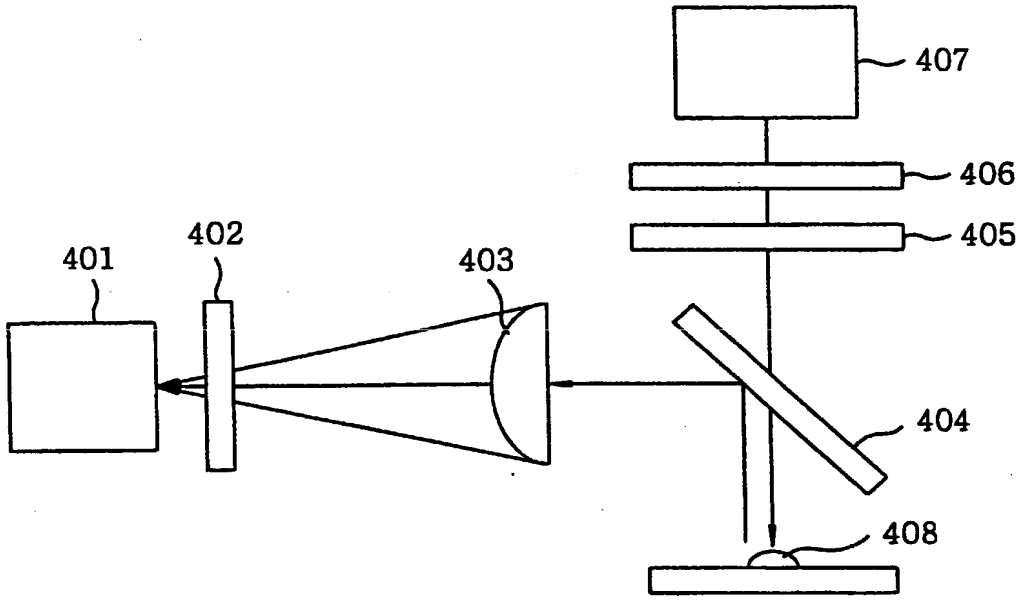
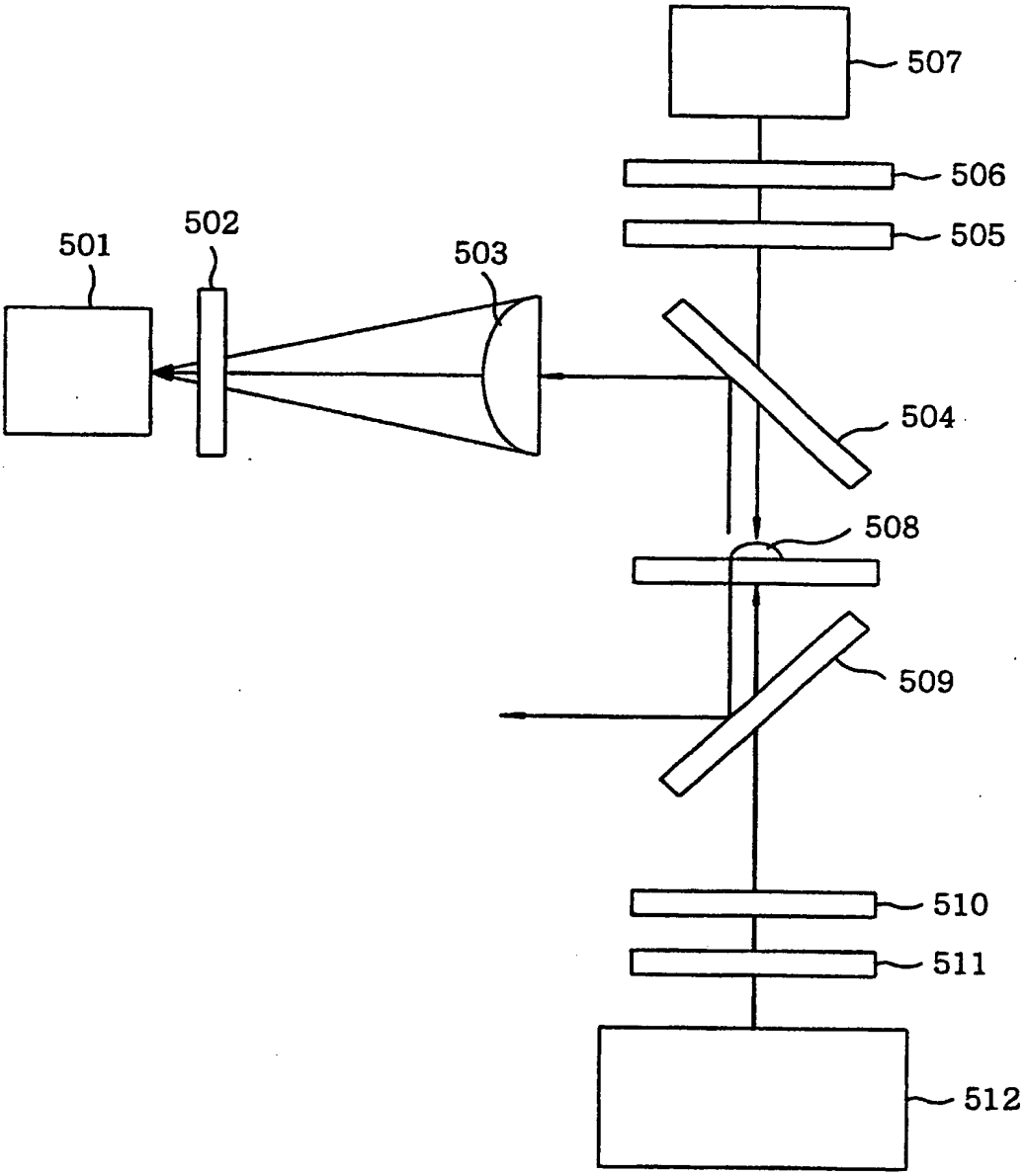


FIG.5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/02030

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 G01N 21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korea patents applications for inventions since 1975, Korea utility models and applications for utility model since 1975
Japanese utility models and applications for utility model since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

NPS, "microchip", "biochip", "fluorescent", "read", "scan"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,271,042 B1 (Alpha Innotech Corp.) 7 AUG. 2001 See the whole document	1-8
Y	JP 2001-194310 A (YOKOGAWAELECTRICCORP) 19 JUL. 2001 See the whole docunent	1-8
A	JP 2001-194309 A (YOKOGAWAELECTRICCORP) 19 JUL. 2001 See the whole document	1-8
A	JP 2000-321206 A (HITACHISOFTWAREENGCOLTD) 20 NOV. 2000 See the whole document	1-8

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☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 FEBRUARY 2003 (18.02.2003)	20 FEBRUARY 2003 (20.02.2003)

Name and mailing address of the ISA/KR	Authorized officer
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